Cucurbitane Glycosides from Unripe Fruits of Siraitia grosvenori

Dianpeng Li, Tsuyoshi Ikeda, Toshihiro Nohara, Jinlei Liu, Yongxin Wen, Tatsunori Sakamoto, and Gen-Ichiro Nonaka

Faculty of Medical and Pharmaceutical Science, Kumamoto University; 5–1 Oe-honmachi, Kamamoto 862–0973, Japan;
Guanxi Institute of Botany, Chinese Academy of Science; Guilin 541006, China; Sakamoto Limestone Industrial Co., Ltd.; 273–1 Tamana, Kumamoto 865–0013, Japan; and Nonaka Usaien Pharmaceutical Co., Ltd.; 1–4–6 Zaimoto, Saga 840–0055, Japan.

Received February 3, 2007; accepted April 10, 2007

Studies on the constituents of the unripe fruits of Siraitia grosvenori led to the isolation of three new cucurbitane triterpene glycosides, 11-oxomogroside III (10), 11-dehydroxymogroside III (11), and 11-oxomogroside IV A (12). Their structures were determined on the basis of detailed analyses of 1D, 2D-NMR spectroscopic methods. All of the compounds isolated from the unripe fruits of S. grosvenori were tested for cytotoxic activities against tumor cells, HCT-116 and SMMC-7721.

Key words Siraitia grosvenori; Lo Han Kuo; cucurbitane-glycoside; unripe fruit; cytotoxic activity

Siraitia grosvenori Swingle (formerly Momordica grosvenori Swingle), a traditional Chinese fruit, belongs to the family cucurbitaceae and has been used as a pulmonary demulcent and emollient for the treatment of dry cough, sore throat, dire thirst, and constipation in folk medicine. A number of cucurbitane triterpene saponins from the ripe fruits were previously obtained. On the basis of its characteristic that the ripe fruit is very sweet, its extract is commercially utilized as a sweet component in sugar substitute; it has a characteristic that the ripe fruit is very sweet, its extract is commercially utilized as a sweet component in sugar substitute; it has been used as a pulmonary demulcent and emollient for the treatment of dry cough, sore throat, dire thirst, and constipation in folk medicine.

To whom correspondence should be addressed. e-mail: none@gpo.kumamoto-u.ac.jp

© 2007 Pharmaceutical Society of Japan

© 2007 Pharmaceutical Society of Japan

Fig. 1. The Structure of 2, 10—12

Notes

three anomic protons indicated all β-glycosidic linkages. When the 1H- and 13C-NMR spectra of 10 were compared with our reported compound 11-oxomogroside II E (Fig. 1), 11 signals ascribable to the aglycone were identical. Moreover, the 13C-NMR data were analogous with the reported data of cucurbitane triterpenoid. The above observation led to the identification of the aglycone as 11-oxomogrol. 13) The 13C-NMR glycosylation shift at δ 87.1 and 92.5 toward downfield suggested that the sugar moiety was attached to C-3 and C-24 of the aglycone. This was further confirmed by heteronuclear multiple bond connectivity (HMBC) correlation of anomic protons (Fig. 2). By comparing the 1H- and 13C-NMR signals due to the sugar moieties of 10 with those of 11-oxomogroside II E, one more glucosyl signal increased in the sugar moiety of 10, an anomic proton at δ 4.84 (1H, d, J=8.2 Hz) and carbon signals at δ 104.8, 75.4, 78.7, 71.8,
ion of 11 was smaller by 14 mass units than that of 10. The aglycone structure of 11 was further confirmed by HMBC and COSY correlations (Fig. 3). Consequently, the structure of 11 was characterized as 3β,24,25-tri-hydroxy-(24R)-cucurbit-5-en-3-O-β-D-glucopyranoside-24-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside] (11-dehydroxymogroside III).

Compound 12, a white amorphous powder, [α]D = −16.9° (MeOH), showed a quasi-molecular ion peak at m/z 1144.1212 [M+Na]+ in the positive HR-FAB-MS, corresponding to the molecular formula C54H90O24Na, which was supported by the 13C-NMR spectrum and its DEPT measurement (Table 1). The 13C-NMR spectrum displayed signals due to eight methyls, twelve methylenes, twenty-seven methines, and seven quaternary carbons. The 1H-NMR spectrum (Table 1) of 12 exhibited signals due to eight tertiary methyls, twelve methylenes, twenty-seven methines, and seven quaternary carbons. The 1H-NMR spectrum of 12 showed signals due to eight methyls, twelve methylenes, twenty-seven methines, and seven quaternary carbons. The 13C-NMR spectra of 12 showed signals ascribable to the aglycone moiety (Table 1). Acid hydrolysis of 12 yielded only D-glucose. The coupling constants J = 7.9, 7.4, 7.4 Hz of the respective anomeric protons indicated all β-glycosidic linkages. When the 1H- and 13C-NMR spectra of 12 were compared with those of 10, signals ascribable to the aglycone were identical. On the other hand, one more glucosyl signal clearly occurred in the sugar moiety of 12, that is, to bear an anomeric proton at δ 5.14 (1H, d, J = 7.9 Hz) and the carbon signals at δ 105.5, 75.5, 78.1, 71.9, 77.9, 62.9, respectively, in the HMQC. Moreover, it showed signals due to four anomeric protons at δ 4.95 (1H, d, J = 7.9 Hz), 4.88 (1H, d, J = 7.4 Hz), 4.83 (1H, d, J = 7.4 Hz), and 5.14 (1H, d, J = 7.9 Hz) along with signals at δ 4.01, 4.19, 4.16, 3.92, 3.96, 4.89; 4.03, 4.21, 4.18, 3.95, 4.36, 4.51; 4.02, 4.16, 4.20, 4.18, 4.34, 4.94, and 4.04, 4.17, 4.22, 4.19, 4.34, 4.50 (each 1H), which correlated with the carbon signals at δ 106.3, 75.1, 78.5, 72.2, 76.4, 70.4; 104.9, 75.4, 78.7, 72.2, 78.4, 62.6; 106.9, 75.3, 78.6, 71.5, 77.3, 70.5; and 105.5, 75.5, 78.1, 71.9, 77.9, 62.9, respectively, in the HMQC. The coupling constants J = 7.9, 7.4, 7.4 Hz of the respective anomeric protons indicated all β-glycosidic linkages. When the 1H- and 13C-NMR spectra of 12 were compared with those of 10, signals ascribable to the aglycone were identical. On the other hand, one more glucosyl signal clearly occurred in the sugar moiety of 12, that is, to bear an anomeric proton at δ 5.14 (1H, d, J = 7.9 Hz) and the carbon signals at δ 105.5, 75.5, 78.1, 71.9, 77.9, 62.9, corresponding to a terminal β-glucopyranosyl moiety (Glc-IV) attached at Glc-II. The linkage site of Glc-IV was determined to be at C-6 of Glc-II, which was shifted toward downfield from δ 63.2 to 70.5. This linkage was confirmed by the HMBC (Fig. 4) experiment, which showed a long-range correlation between the signals at δ 5.14 (1H, d, J = 7.9 Hz, Glc-IV anomeric H) and 70.5 (Glc-II C-6), and also by the NOESY (Fig. 4) corre-
relation between the signals at δ 5.14 (1H, d, J = 7.9 Hz, Glc-IV anemic H) and δ 4.34, 4.94 (each 1H, Glc-II H-6). Hence 12 was formulated as 3β,24,25-tri-hydroxy-(24R)-cucurbit-5-en-11-one-3-0-[β-D-glucopyranosyl(1—6)-β-D-glucopyranosyl]-24-O-[β-D-glucopyranosyl(1—6)-β-D-glucopyranosyl] (11-oxomogroside IV).

Recently, physiologial functions of *S. grosvenori* and its components have received considerable attention, and some interesting findings have been reported. For example, mogroside V has been shown to have inhibitory effects on the initiation and promotion of cancer. It might be valuable as a chemopreventive agent against chemical carcinogenesis.12) Mogroside IV (226) and mogroside III (263) were also found to have inhibitory effects against HCT-116 colon cancer cells and SMMC-7721 hepatoma cells (14). Mogroside II (217) was found to have a strong inhibitory effect and promotion of cancer. It might be valuable as a chemopreventive agent against chemical carcinogenesis.12) Mogroside III (211) and Mogroside I (232) were also found to have inhibitory effects against HCT-116 colon cancer cells and SMMC-7721 hepatoma cells.14) Mogroside I was found to have a strong inhibitory effect on maltase activity.15) In our study, all cucurbitane components have received considerable attention, and some interesting findings have been reported. For example, mogroside V was found to have a strong inhibitory effect and promotion of cancer. It might be valuable as a chemopreventive agent against chemical carcinogenesis.12) Mogroside IV (226) and mogroside III (263) were also found to have inhibitory effects against HCT-116 colon cancer cells and SMMC-7721 hepatoma cells.14) Mogroside II (217) was found to have a strong inhibitory effect and promotion of cancer. It might be valuable as a chemopreventive agent against chemical carcinogenesis.12)

Experimental

General Experimental Procedures

Optical rotations were measured by P-1010 polarimeter (JASCO, Japan) at 25 °C. TLC was performed on precoated silica gel 60 F254 plate (Merck), and detection was by spraying 10% aq. H2SO4. Column chromatographies were carried out on Kiesel gel (40—100 mesh and 230—400 mesh, Kanto Chem.). Diaion HP-20 (Mitsubishi Chemical Ind.), Sephadex LH-20 (25—100 mm, Pharmacia Fine Chemicals), Wakogel 50 C18 (36—212 mm, Wako Pure Chemical Industries Ltd.), Chromatex ODS (30—50 μm, Fuji Silysia Chemical Ltd.). FAB-MS were measured by JEOL JMS-DX300HF spectrometer (Xe atom beam, accel. voltage 2—3 kV, matrix glycerol), 200—300 mA. NMR spectra were recorded at 500 MHz for 1H and 125 MHz for 13C by JNC-A500 spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as internal standard. Standard pulse sequences were employed for DEPT, HMBC, and HMB experiments. NOE spectra were measured with mixing times of 600 ms. Plant Material

Unripe fruits of *S. grosvenori* (40—50 days of growing) were obtained from Lingui county, Guilin city of Guangxi province, China, in October 2004 and identified by Professor Wei Huanan. A voucher specimen (SG05820) of the plant is deposited at the Herbarium of Guangxi Institute of Botany, China.

Extraction and Isolation

Fresh unripe fruits (5 kg) of *S. grosvenori* were extracted with methanol (8:1:3) at room temperature for 10 days. The extract was evaporated under reduced pressure to afford methanol extract (205 g). The extract was chromatographed on Diaion HP-20, with successive elution with H2O and methanol 30%, 80%, and 100%. The 80% methanol eluate (30.5 g) was subjected to silica gel column chromatography with CHCl3—MeOH—H2O (8:2:0.2, v/v), followed by further purification with Sephadex LH-20 (30% MeOH) and to Wakogel C18 column chromatography (50—60% MeOH) to afford 10 (91.6 mg) and 11 (66.9 mg). Fraction 6 (320 mg) was repeatedly subjected to silica gel column chromatography with CHCl3—MeOH—H2O (8:2:0.2, v/v), followed by further purification with Sephadex LH-20 (30% MeOH) and to Wakogel C18 column chromatography (50—60% MeOH) to afford 10 (91.6 mg) and 11 (66.9 mg). Fraction 6 (320 mg) was repeatedly subjected to silica gel column chromatography with CHCl3—MeOH—H2O (8:2:0.2, v/v), followed by further purification with Chromatex ODS (55—65% MeOH) to give 12 (9.4 mg).

11-Oxomogroside III (10): A white amorphous powder, [α]D +56.7° (c = 0.1, MeOH). Positive FAB-MS (m/z): 984 [M+Na]+. Positive HR-FAB-MS (m/z): 983.5261 [M+Na]+ (Calcd for C48H80O19Na, 983.5192). 1H- and 13C-NMR (in pyridine-d5) given in Table 1.

11-Dehydroxymogroside III (11): A white amorphous powder, [α]D +7.5° (c = 0.2, MeOH). Positive FAB-MS (m/z): 970 [M+Na]+. Positive HR-FAB-MS (m/z): 969.5486 [M+Na]+ (Calcd for C43H77O19Na, 969.5399). 1H- and 13C-NMR (in pyridine-d5) given in Table 1.

11-Oxomogroside IV (12): A white amorphous powder, [α]D −16.9° (c = 0.2, MeOH). Positive FAB-MS (m/z): 1144 [M+Na]+. Positive HR-FAB-MS (m/z): 1144.1212 [M+Na]+ (Calcd for C48H80O19Na, 1144.1085). 1H- and 13C-NMR (in pyridine-d5) given in Table 1.

Acid Hydrolysis of 10, 11, and 12

A solution of 10, 11, and 12 (5.0 mg, 4.5 mg and 2.5 mg, respectively) in 0.5% HCl was heated under reflux for 2 h. The reaction mixture eluted with H2O and MeOH successively was subjected to Amberlite IRA-400. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, COSMOSIL Sugar-α, 4.6 mm i.d. ×250 mm (Nacalai Tesque, Co., Ltd., Tokyo, Japan); detector, JASCO OR-2000; pump, JASCO PU-2080; mobile solvent: 80% CH3CN; flow rate, 0.8 ml/min; column oven, Co-2060 plus; column temperature, 35 °C. Identification of D-glucose in the aqueous layer was carried out with mixing times of 600 ms. The reaction mixture eluted with H2O and MeOH successively was subjected to Amberlite IRA-400. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, COSMOSIL Sugar-α, 4.6 mm i.d. ×250 mm (Nacalai Tesque, Co., Ltd., Tokyo, Japan); detector, JASCO OR-2000; pump, JASCO PU-2080; mobile solvent: 80% CH3CN; flow rate, 0.8 ml/min; column oven, Co-2060 plus; column temperature, 35 °C. Identification of D-glucose in the aqueous layer was carried out with mixing times of 600 ms.

Table 1. Cytotoxic Activities of Compounds 1—12 against HCT-116 Colon Cancer Cells and SMMC-7721 Hepatoma Cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>SMMC-7721</th>
<th>HCT-116</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-Hydroxy-11-oxomogroside I</td>
<td>295</td>
<td>624</td>
</tr>
<tr>
<td>11-Oxomogroside II</td>
<td>390</td>
<td>309</td>
</tr>
<tr>
<td>11-Oxomogroside I</td>
<td>211</td>
<td>630</td>
</tr>
<tr>
<td>Mogroside II</td>
<td>226</td>
<td>657</td>
</tr>
<tr>
<td>Mogroside III</td>
<td>263</td>
<td>401</td>
</tr>
<tr>
<td>Mogroside IV</td>
<td>232</td>
<td>863</td>
</tr>
<tr>
<td>Mogroside V</td>
<td>357</td>
<td>465</td>
</tr>
<tr>
<td>Kaempferol 7-α-L-rhamnopyranoside</td>
<td>115</td>
<td>127</td>
</tr>
<tr>
<td>Kaempferol 3,7-α-L-dirhamnopyranoside</td>
<td>250</td>
<td>331</td>
</tr>
<tr>
<td>11-Oxomogroside III</td>
<td>290</td>
<td>260</td>
</tr>
<tr>
<td>11-Dehydroxymogroside III</td>
<td>217</td>
<td>945</td>
</tr>
<tr>
<td>11-Oxomogroside IV</td>
<td>288</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

a) Concentration inhibiting 50% of cell growth (IC50). b) Not determined.
styrene treated) and incubated for 24 h. Test compound solutions in 10% dimethyl sulfoxide (DMSO) in various concentrations (50, 100, 200, 400 μg/ml) were prepared and 50 μl of the test solution or 10% DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. Then, 5% MTT was added and the plate was incubated for another 4 h. Thereafter, 150 μl of DMSO was added and the absorbency was read on a microplate reader (ELISA, Anthos 2010, Anthos Labtec Instruments Inc.) at 492 nm. A dose–response curve was plotted for each compound, and the concentrations giving 50% inhibition of cell growth (IC50) were calculated (see Table 2).

Acknowledgments The authors wish to thank NPO Tsukushi Scholarship and Research Foundation for financial support to Dianpeng Li.

References